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LUPEOL AND LAURIC ACID ISOLATED FROM ETHYL ACETATE STEM EXTRACT OF *Justicia secunda* AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

The method of cold maceration was used in the extraction of *Justicia secunda* starting with a non-polar (hexane) to a more polar solvent. The crude extracts of hexane, ethyl acetate, acetone, and methanol from the stem of were obtained using the polarity guided cold extraction method. Lupeol and Lauric acid are two well-known compounds that were discovered when a portion of the ethyl acetate extract of *J. secunda* was subjected to spectroscopic (1H NMR) structural elucidation. Based on the presence of Lupeol and Lauric acid in *J. secunda* stem, the plant could be a viable source of antimicrobial agents in the near future. The results of the antimicrobial activity obtained from the fractions of the Stem inhibited or exhibited activity against *Methicillin resist Staph aureus, Vancomycin resistant enterococci, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Helicobacter pylori, Campylobacter fetus, Proteus mirabilis, Pseudomonas aeruginosa, Candida tropicalis, and Candida krusei*. The plant *J. secunda* traditionally employed in the treatment of anemic circumstance, blood boost, wound healing, abdominal pain and fertility issues. The overall results confirm the significance of the use of the plant in traditional medicinal treatment of anamic circumstances, blood boost, wound healing etc, in line with reported claims because of the presence of isolated compounds.

Keywords: *Jussticia secunda* stem isolates or fractions, Column chromatography and Thin Layer Chromatography, Lupeol, Lauric acid, ^{1}H NMR, structural elucidation, antimicrobial activities.

INTRODUCTION

Due to their numerous benefits to humanity, particularly their use in the food and pharmaceutical industries, a wide variety of medicinal plants found all over the world have caught the attention of researchers. The presence of bioactive components that produce definite physiological action in the human body was the cause of their medicinal and pharmacological properties [1]. Bioactive components are chemical compounds or substances isolated from living organism. The chemistry of the bioactive components includes their biosynthesis, extraction, identification, quantification, structural elucidation, physical and chemical properties and reactions They are produced by the pathway of primary or secondary metabolism [2]. The study of bioactive components discovered by laborious chemical methods has become a major area of research in organic chemistry, biological sciences and has led to the isolation of thousands of different structures, mostly extracted from plants and more recently from microorganisms, with the animal kingdom contributing rather sparsely to the total [3]. The upsurge in techniques involved in the isolation and identification of novel natural compounds from

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plant materials have made the discovery of novel compounds which have the potential to become drugs more feasible. Isolation of bioactive compounds from different parts of plants is performed using chromatographic techniques such as Thin Layer Chromatography (TLC), Preparative Thin Layer Chromatography (PTLC), Column Chromatography (CC), Medium Pressure Liquid Chromatography (MPLC) and High Performance Liquid Chromatography (HPLC) amongst others [4]. Structure elucidation provides the molecular structures of compounds through the use of techniques such as Nuclear Magnetic Resonance (NMR) and Mass Spectrometry(MS) analysis [5]. *Justicia secunda* a perennial herbal plant, which belongs to the Acanthaceae family [6]. The plant is widely found in Africa, Asia and the West Indians. Due to their numerous applications, the plants have different names in various regions. There are several documented reports on the medicinal values of this plant and they are traditionally employed in the treatment of anemic circumstance, wound healing, abdominal pain and fertility issues [7]. Biochemical profiling of the plant revealed the presence of antioxidant [8], antimicrobial [9] anti-sickling, haematonic, antihypertensive, anti-inflammatory and other biological activities [7] [10]. To the best of our knowledge, there has not been report on any isolated pure compound and bioactivities of this specie *J. secunda*. Here, we are reporting the isolated compounds (Lupeol and Lauric acid), and antimicrobial properties of the stem extracts of *J. secunda* in Wukari, Taraba State,Nigeria.

MATERIALS AND METHODS

Sample Collection and Preparation

The stem of *Justicia secunda* were collected from their natural habitat in Wukari Local Government Area of Taraba State, Nigeria. The plants were taken to the Department of Forestry and Wild Life Sciences, Federal University Wukari for identification. The fleshly collected stems of *J. secunda* was washed with distilled water, cut into smaller pieces then air-dried in a ventilated room for three weeks. After drying, the stems were pound into powder form with a mortar and a pestle. The powdered plant materials were reserved in sealed containers for extraction. The method of cold maceration was used in the extraction by serial exhaustive extraction method which involves successive extraction with solvents of increasing polarity from a non-polar (hexane) to a more polar solvent to ensure that a wide polarity range of compound could be extracted. The plant material (234 g) was macerated in n-Hexane (2.5 L) using a Winchester bottle for 48 Hrs. The extract was filtered into a glass jar using a Whatman number 1 filter paper. The extract was concentrated in vacuo on a rotatory evaporator. The concentrate was air-dried in a fumehood until all trace of solvent was removed. The hexane extract (3.5 g) was, however, rather oily and was therefore discontinued. The marc was aired to remove solvent traces prior to extraction with ethyl

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acetate. The marc from the n-Hexane maceration was macerated in Ethyl acetate (2.5 L) using a Winchester bottle for 48 Hrs. The extract was filtered into a glass jar using a Whatman number 1 filter paper. The extract was concentrated in vacuum a rotatory evaporator. The concentrate was air-dried in a fumehood until all trace of solvent was removed. The extract was coded JSS (*Justicia secunda* stem) and set aside for column chromatography. The marc was aired to remove solvent traces prior to extraction with methanol.

Isolation of Compounds

The procedures for isolation and purification of the components were thin layer chromatography and column chromatography. Dried extract of the sample was extracted with hexane and ethyl acetate. The extracts were combined (based on similarity on TLC) and subjected to column chromatography using silica gel in a glass column. The column was packed wet in a hexane: ethyl acetate (90:10) mixture and eluted with ethyl acetate in hexane gradient starting with 10 % ethyl acetate in hexane and increasing the amount of ethyl acetate by 10% until 100%ethyl acetate yielding 10-ml vials. Further elution with ethanol in ethyl acetate starting with ethyl acetate: ethanol (90:10) mixture. Plant material (1.66 g) was reconstituted in ethyl-acetate and adsorbed onto silica gel (3g), in vacuo, until a free-flowing powder was obtained. A slurry of silica gel was prepared thus: Silica gel (50g) was dispersed into hexane(200mL) and stirred with a glass rod. The slurry was introduced into a glass column (4.5cm by 47cm). The column was gradiently ran [Hex (95%) in Ethylacetate→Hex (50%)]. (200 mL) was used for the column. Fractions (95 of 20 Mleach) were collected. The fractions were designated JS-1- JS-95 and subjected to Thin Layer Chromatography (TLC) and similar fractions were pooled together. Fraction JS-35 (JS-35.10fid) gave a crystal orange colour while JS-31 (JS-31.10fid) was a colourless crystal. This was stored at room temperature for spectroscopic analysis. Both fractions JS-35.10fid (X) and JS-31.10fid (Y) were characterized using proton Nuclear Magnetic Resonance (1H-NMR).

Antimicrobial Activities of the Fractions

The fractions (JS-35.10fid (X) and JS-31.10fid (Y)) were tested for antibacterial and antifungal activities using methods described by [11] with modification. The antimicrobial activities of JS-35.10fid (X) and JS-31.10fid (Y)were determined using some pathogenic microbes that were obtained from the department of medical microbiology ABU teaching hospital Zaria. The test organisms were collected from the Department of Medical Microbiology ABU teaching hospital Zaria. *Justicia secunda* stem isolates was tested against eleven test organisms such as *Methicillin resist Staph aureus, Vancomycin resistant enterococci, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Helicobacter pylori, Campylobacter fetus, Proteus mirabilis,*

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Pseudomonas aeruginosa, Candida tropicalis, and Candida krusei.

Given amount of 0.001mg of the compound was weighed and dissolved in 10mls of DMSO to obtain a concentration of 100µg/ml. This was the initial concentration of the compound used to determine its antimicrobial activities. Diffusion method was the method used for screening the compound. Mueller Hinton agar was the medium used as the growth medium for the microbes. The medium was prepared according to the manufacturer instructions sterilized at 121ºC for 15mins, poured into the sterile petri dishes and was allowed to cool and solidify. The sterilized medium was seeded with 0.1ml of the standard inoculums of the test microbe, the inoculum was spread evenly over the surface of the medium by the used of sterile swab. By the use of a standard cork borer of 6mmin diameter a well was cut at the center of each inoculated medium. 0.1ml of solution of the compound of the concentration of 100µg/ml was then introduced into the well on the inoculated medium. Incubation was made at 370C for 24hrs, after which the plates of the medium were observed for the zone of inhibition of growth, the zone was measured with a transparent ruler and the result recorded in millimeter.

Minimum Inhibitory Concentration (MIC) Assay

The minimum inhibition concentration of the compound was determined using the broth dilution method as described by [11], Mueller Hinton broth was prepared, 10mls was dispensed into test tubes and was sterilized at 1210c for 15mins, the broths were allowed to cool.MC-farland's turbidity standard scale number 0.5 was prepared to give solution. Normal saline was prepared, 10mls was dispensed into sterile test tube and the test microbe was inoculated and incubated at 370c for 6hrs.Dilution of the microbe was done in the normal Saline until the turbidity marched that of the MC-farland's scale by visual comparison at this point the test microbe has a concentration of about1.5Xc/ml. Two-fold serial dilution of the compound was done in the sterile broth to obtain the concentrations of 100µg/ml,50µg/ml,25µg/ml,12.5µg/ml, and 6.25µg/ml. The initial concentration was obtained by dissolving 0.001mg of the compound in10mls of the sterile broth. Having obtained the different concentrations of the

compound in the sterile broth, 0.1ml of the test microbe in the normal saline was then inoculated into the different concentrations, incubation was made at 37^{0}C for 24hr, after which the test tubes of the broth were observed for turbidity(growth) the lowest concentration of the compound in the sterile broth which shows no turbidity was recorded as the minimum inhibition concentration.

Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

MBC/MFC were carried out to determine whether the test microbes were killed or only

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their growth was inhibited as described by [11]. Mueller Hinton agar was prepared sterilized at 121oc for 15mins, poured into sterile petri dishes and was allowed to cool and solidly. The contents of the MIC in the serial dilutions were then sub cultured onto the prepared medium, incubation was made at 370c for 24hrs, after which the plates of the medium were observed for colony growth, MBC/MFC were the plates with lowest concentration of the compound without colony growth.

RESULTS AND DISCUSSION

Spectra Data of the Isolated Compounds

Results of ^{1}HNMR for Compound **X** (JS-35.10.fid) and Compound **Y** (JS-31.10.fid) experimental, in comparison with data from previous literature are presented in Table 1.

Table 1. ^{1}HNMR Spectra Data of Compound X (JS-35.10.fid)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.68 (d, *J* = 2.5 Hz, 1H), 4.56 (dd, *J* = 2.7, 1.4 Hz, 1H), 3.19 (dd, *J* = 11.1, 5.1 Hz, 1H), 2.42 – 2.29 (m, 2H), 1.96 – 1.85 (m, 1H), 1.69 – 1.67 (m, 5H), 1.02 (s, 3H), 0.96 (s, 3H), 0.94 (s, 3H), 0.82 (s, 3H), 0.78 (s, 3H), 0.75 (s, 3H), 0.70 – 0.66 (m, 1H).

Position of Proton (H)	Experimental ^{1}H Chemical shift δ (ppm), J (Hz)	Literature ^{1}H Chemical shift δ (ppm)	
		[12]	[13]
1			
2	1.67,m	1.64	1.65
3	3.19,dd	3.23, m	3.16
4	-		
5	0.68,m	0.73, m	0.71, t
6		1.42, m	1.53
7			
8	-		
9		1.23, s	
10	-		
11			
12			
13			
14	-		
15			1.10, d
16			
17	-		
18		1.34, s	1.43, t
19	2.36,m	2.40, m	2.43, m
20			
21	-		1.05

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22			1.19,1.37
23	0.96,s	0.97, s	0.96, s
24	0.75,s	0.79, s	0.79
25	0.82,s	0.82, s	0.89
26	1.02,s	1.03, s	1.13
27	0.94,s	0.94, s	1.01
28	0.78,s	0.86, s	0.83
29	4.68,d	4.70	4.69
	4.56,dd	4.59	b. 4.58
30	1.91,m	1.71, s	1.68, s

Table 2. ^{1}HNMR Spectra Data of Compound Y (JS-31.10.fid)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 2.35 (t, *J* = 7.5 Hz, 1H), 1.72 – 1.58 (m, 3H), 1.25 (s, 13H), 0.90 – 0.84 (m, 3H).

Position of Proton (H)	Experimental ^{1}H Chemical shift δ (ppm), J (Hz)	Literature ^{1}H Chemical shift δ (ppm)	
		[14]	[15]
1	-		
2	2.35,t	2.28,m	2.30,m
3	1.63,m	1.63,m	1.52,m
4	1.25,s	1.32,m	1.29,m
5	1.25,s	1.32,m	1.26,m
6	1.25,s	1.31,m	1.26,m
7	1.25,s	1.31,m	1.26,m
8	1.25,s	1.31,m	1.26,m
9	1.25,s	1.31,m	1.29,m
10	1.30,s	1.30	1.29,m
11	1.31,s	1.37,m	1.31,m
12	0.88,m	0.99,t	0.88,t

Antimicrobial Activities Results of the Stem Isolates of Justicia secunda

Activity of the isolates from the stem of *Justicia secunda* was tested on eleven (11) clinical isolates. The results obtained are summarized in Table 3.

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Table 3. Antimicrobial activities of purified fractions of JS1, JS2 compound and Control.

Test Organism	JSF1	JSF2	Ciprofloxacin	Fluconazole
Methicillin Resist Staph aureus	S	S	R	R
Vancomycin resist enterococci	R	S	R	R
Staphylococcus aureus	S	R	S	R
Escherichia coli	S	S	S	R
Klebsiella pneumonia	R	R	R	R
Helicobacter pylori	S	S	S	R
Campylobacter fetus	S	R	R	R
Proteus mirabilis	R	S	S	R
Pseudomonas aeruginosa	R	R	R	R
Candida tropicalis	S	S	R	S
Candida krusei	S	S	R	S

Keywords: R = Resistance, **S =** Sensitive**, JSF**= *Justicia secunda* stem fraction

Zone of inhibition of the Compoundof the Stem Isolates of Justicia secunda

Activity of the isolates from the stem of *Justicia secunda* was tested on eleven (11) clinical isolates. The measured zone of inhibition of the pathogens by the stem isolates are summarized in table 4 below.

Table 4. Zone of inhibition of the Compound of the Isolates of *Justicia secunda* Stem extracts against the test Micro Organism.

Test Organism	JSF1	JSF2	Ciprofloxacin	Fluconazole
Methicillin Resist Staph aureus	25	27	0	0
Vancomycin resist enterococci	0	22	0	0
Staphylococcus aureus	23	0	35	0
Escherichia coli	27	26	37	0
Klebsiella pneumonia	0	0	0	0
Helicobacter pylori	24	28	31	0
Campylobacter fetus	21	0	0	0
Proteus mirabilis	0	27	30	0
Pseudomonas aeruginosa	0	0	0	0
Candida tropicalis	21	25	0	32
Candida krusei	23	26	0	30

Keywords: JSF= *Justicia secunda* stem Fraction

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Minimum Inhibitory Concentration of the Compounds of the Stem Isolates of Justicia secunda

The measured minimum inhibitory concentration (MIC) of the isolates from the stem of *Justicia secunda* against the test organisms are summarized in Table 5.

Table 5. Minimum Inhibitory Concentration of the Compound of the Stem Isolates against the Test Microorganism

Test Organism	JSF1					JSF2				
	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml
Methicillin Resist Staph aureus	-	-	0*	+	++	-	-	-	0*	+
Vancomycin resist enterococci						-	-	0*	+	++
Staphylococcus aureus	-	-	0*	+	++					
Escherichia coli	-	-	-	0*	+	-	-	0*	+	++
Klebsiella pneumonia										
Helicobacter pylori	-	-	0*	+	++	-	-	-	0*	+
Campylobacter fetus	-	-	0*	+	++					
Proteus mirabilis						-	-	-	0*	+
Pseudomonas aeruginosa										
Candida tropicalis	-	-	0*	+	++	-	-	0*	+	++
Candida krusei	-	-	0*	+	++	-	-	0*	+	++

Keywords: **-** = No turbidity (no growth), **O*** = MIC, + = Turbid (light growth), ++ = Moderate turbidity, +++ = High turbidity

Minimum Bactericidal/Fungicidal concentration of the Compounds from stem isolates of Justicia secunda.

The measured minimum inhibitory concentration (MIC) of the isolates from the stem isolates of *Justicia secunda* against the test organisms are summarized in Table 6.

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Table 6. Minimum Bactericidal/Fungicidal Concentration of the Compound of the Stem Isolates Against the Test Microorganism.

Test Organism	JSF1					JSF2				
	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml
Methicillin resist Staph aureus	-	0*	+	++	+++	-	0*	+	++	+++
Vancomycin resist enterococci						0*	+	++	+++	+++
Staphylococcus aureus	0*	+	++	+++	+++					
Escherichia coli	-	0*	+	++	+++	-	0*	+	++	+++
Klebsiella pneumonia										
Helicobacter pylori	-	0*	+	++	+++	-	-	0*	+	++
Campylobacter fetus	0*	+	++	+++	+++					
Proteus mirabilis						-	0*	+	++	+++
Pseudomonas aeruginosa										
Candida tropicalis	0*	+	++	+++	+++	-	0*	+	++	+++
Candida krusei	0*	+	++	+++	+++	-	0*	+	++	+++

Keys: - =No Colony Growth, **O*** = MBC/MFC, + = Scanty colonies growth, ++ = Moderate colonies growth, +++ = Heavy colonies growth**.**

Structural elucidation

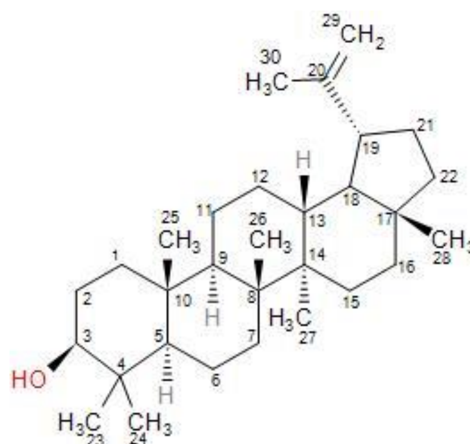
The structural elucidation was done by spectroscopic method (1H NMR) and was carried out on the purified extracts. Nuclear magnetic resonance (NMR) is a spectroscopic method that is important to organic chemists. The results revealed two compounds identified as Lupeol and Lauric acid.

Compound X

The 1H-NMR spectra of the isolated compound showed a characteristic pattern of a triterpenoid, comparison of the spectra data of JS-35.10.fid (**X**) and that of lupeol from literature are similar. In the proton NMR spectrum of JS-35.10.fid (**X**), olefinic protons of H-29 showed signals at δH4.69ppm (1H, d) and 4.58 respectively, this supported the double bond between methylene

carbon (C– 29) and quaternary carbon (C – 20). It also revealed the presence of seven tertiary methyl protons at δ 0.75, 0.78, 0.82, 0.94, 0.96, 1.02 and 1.71 ppm (integrated for 3-H, each, s, CH_3).

One such agent which has gained wide attention of medical professionals, pharmaceutical marketers and researchers all around the world, is a dietary triterpene known as Lupeol. The chemical formula of Lupeol is $C_{30}H_{50}O$ and its melting point is 215–216 °C. The molecular formula depicts the presence of six degrees of unsaturation, out of them one can be satisfied by an olefinic function. The presence of seven methyl singlets and an olefinic function in the 1H-NMR spectrum revealed that Lupeol is pentacylic triterpenoidal type in nature. Lupeol, is found in vegetables such as white cabbage, pepper, cucumber, tomato, in fruits as olive, fig, mango, strawberry, red grapes etc [16]. Lupeol has been shown to exhibit various pharmacological activities under *in vitro* and *in vivo* conditions. These include its beneficial activity against inflammation, cancer, arthritis, diabetes, heart diseases, renal toxicity and hepatic toxicity [17].



Structure of Lupeol

Compound Y

The 1H-NMR spectrum of Compound Y (JS-31.10.fid) exhibited 13 proton resonates. A doublet proton signal was observed at δ 2.35, δ 1.63, δ 1.25, δ 1.25, δ 1.25, δ 1.25, δ 1.25, δ 1.25, δ 1.30, and δ 1.31, respectively. Indicating the presence of a methylene group of the long chain of Compound Y and was assigned to H-2, H-3, H-4, H-5, H-6, H-7, H-8, H-9, H-10, and H-11. A multiplet proton signal was observed at δ 0.88 (3H, m) which correspond with a methyl group and was assigned to H-12. According to [18] findings, the proton NMR data of Compound Y were largely identical to the NMR signal of dodecanoic acid.

Lauric Acid (Dodecanoic acid), is a compound with a carboxylic group attached to the first carbon's terminal end. It was reported that the acid had virucidal effects on viruses with DNA and RNA envelopes. It was also found to have inactivated enveloped viruses, yeast, fungi, and bacteria. Alpha- and beta-MG make it an active antimicrobial as well. Additionally, the additive

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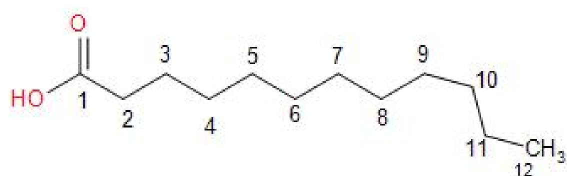
nature of the dodecanoic acid's antimicrobial effects and the importance of its total concentration in virus inactivation are mentioned [15]. Numerous pathogenic organisms have been reported to be inactivated by these antimicrobial compounds, and dodecanoic acid has stronger antiviral properties. Opportunistic infections in HIV-positive individuals are known to be caused by it. It is capable of eliminating harmful pathogens like fungi, viruses, and bacteria. Infants may benefit from this important compound by lowering their risk of developing heart disease and cancer in the future [19].

According to a study that was published in 2014 and was conducted by [20] feeding pregnant mice dodecanoic acid increased the suckling Neonatal pups' resistance to the giardia duodenalis infection. Yamashita reported the discovery of dodecanoic acid compounds from *Helicobacter pylori* that inhibit self-growth [15]



Structure of Lauric Acid

Antimicrobial Results of the Fractions (Isolates) from the Justicia secunda stem

The results of the antimicrobial activity obtained from the fractions (isolates) of the *Justicia secunda* stem as shown in Tables 4, 5 and 6 revealed that all isolates inhibited or exhibited antibacterial and antifungal activities against seven clinical isolates (test organisms). *Justicia secunda* Fraction 1 (JSF1) were active against the following seven clinical isolates: *E. coli* (27mm zone diameter inhibition), *Methicillin resistant staphylococcus* (25mm zone diameter inhibition), *Helicobacter pylori* (24mm zone diameter inhibition), *Staphylococcus aureus* (23mm zone diameter inhibition), *Campylobacter fetus* and *Psuedomonas aeruginosa* (21mm zone diameter inhibition) but did not show any activity against *Vancomycin resistant enterococci*, *Klebsiella pneumonia, Proteus mirabilis* and *Psuedomonas aeruginosa*. *Justiciasecunda* Fraction 2 (JSF2) were active against the following seven clinical isolates: *Helicobacter pylori* (28mm zone diameter inhibition), *Methicillin resistant staphylococcus* and *Proteus mirabilis* (27mm zone diameter inhibition), *E. coli* and *Candida krusei* (26mm zone diameter inhibition), *Candida tropicalis* (25mm zone diameter inhibition) and *Vancomycin resistant enterococci* (22mm zone diameter inhibition) but did not show any activity against *Staphylococcus aureus, Klebsiella pneumonia, Campylobacter fetus*, and *Psuedomonas aeruginosa*. Ciprofloxacin demonstrated the highest activity value against *E. coli* (37mm zone diameter inhibition), *Staphylococcus aureus* (35mm zone diameter inhibition), *Helicobacter pylori* (31mm zone diameter inhibition), and *Proteus mirabilis* (30mm zone diameter inhibition) while Fluconazole demonstrated the highest activity against two clinical isolates; *Candida tropicalis* and *Candida*. The results obtained revealed that

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the plant, *Justiciasecunda* stem isolates can be used in bacteria and fungi treatment.

The MIC and MBC of the fractions (isolates) ranged from 6.25 - 100µg/mlwith *Justicia secunda* fraction 1 (JSF1) demonstrating the lowest values (MIC 12.5µg/ml : MBC 50 µg/ml each) against *E. coli : Methicillin resistant staphylococcus, E. coli, and Helicobacter pylori* respectively while *Justicia secunda* fraction 2 (JSF2) demonstrating the lowest values (MIC 12.5µg/ml : MBC25µg/ml) against *Methicillin resistant staphylococcus* and *Helicobacter pylori* : *Proteus mirabilis* and *Helicobacter pylori* respectively. With almost the fractions (JSF1) demonstrating the highest value (MIC 25µg/ml : MBC 100) against *Methicillin resistant staphylococcus, Staphylococcus aureus, Helicobacter pylori, Campylobacter fetus, Candida tropicalis* & *Candida krusei : Staphylococcus aureus, Campylobacter fetus, Candida tropicalis* & *Candidakrusei* respectively while JSF2 demonstrating the highest values (MIC 25µg/ml) against *Vancomycin resistant enterococci, E. coli, Candida tropicalis* & *Candida krusei* and MBC 50 & 100µg/ml against *Methicillin resistant staphylococcus, Vancomycin resistant enterococci, E. coli, Proteus mirabilis, Candida tropicalis* and *Candida krusei* respectively. Most of the MIC values were lower than the MBC values. The lower MIC value indicates that less plant isolates (fractions) is required for inhibiting growth of the organism; therefore, plants with lower MIC scores are more effective antimicrobial agents. The fact that the plant, *Justiciasecunda* stem fractions or isolates was active against clinical isolates is also an indication that it can be a source of a very potent antimicrobial substances that can be used against drug resistant microorganisms prevalent in hospital environs.

CONCLUSION

The structural elucidation of *Justicia secunda* stem isolates by spectroscopy (1H NMR) revealed two compounds that are known as lupeol and Lauric acid. The data presented here suggest that the fractions of lupeol and Lauric acid can be extracted from *J. secunda*, which is used as due to their anti-inflammatory, antioxidant, cytotoxic, and anticancer properties, lupeol and lauric acid have the potential to treat acute pancreatitis. The obtained antimicrobial activity results revealed that the plant, *J. secunda* stem isolates, can be utilized in the treatment of bacteria and fungi. The traditional healers' claims that the *J. secunda* stem is used to treat some illnesses are supported by the possibility that the plant extracts could be a source for useful drugs. As a result, it is encouraged that these plants continue to be used as traditional medicines.

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